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14. ABSTRACT

BRCA1 coordinates cellular responses to DNA damage. It functions as a co-repressor of GADD45atranscription through interactions with a DNA-binding protein termed ZBRK1. Our goal is to develop a biosensor system to visualize transcription control by ZBRK1 and BRCA1 in single living and/or fixed cells. The rationale is to use integrated DNA binding sites to obtain real time, multiplex-based data. The reportable outcomes for the period are: 1) UAS and ZREarray-bearing plasmids have been constructed; 2) Transient and stable reporter expression have been demonstrated; 3) Stable cell lines with G20, G40 and Z32 are on line for studies;4) Construction of fluorescent GAL4-DBD and ZBRK1 fusion protein has been achieved, and BRCA1derivatives are in progress. When operational, this system will document real time nucleardynamics of ZBRK1/BRCA1-dependent chromatin modification systems, as cells mount transcriptional responses to genotoxins.

15. SUBJECT TERMS
BRCA1; ZBRK1; Breast Cancer, Nuclear Dynamics

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This is a revised report to comply with formatting requirements noted by the reviewer. We apologize for this error and appreciate the opportunity to rectify this mistake in reporting. The data in the PowerPoint presentation is now included in the body of the report.

Introduction:

Families carrying defective *breast cancer 1* (*BRCA1*) genes have a cumulative lifetime risk of breast and ovarian cancer (Hall et al., 1990; Miki et al., 1994; Wooster and Weber, 2003). BRCA1 coordinates cellular responses to DNA damage (Rosen et al., 2003; Zheng et al., 2000a). Functions attributed to BRCA1 include chromatin modification, ubiquitinylation, and transcription regulation. Regarding transcription, BRCA1 has been implicated in both activation and repression (Monteiro, 2000). One notable target is the growth arrest and DNA damage-inducible gene, GADD45 (Harkin et al., 1999; Jin et al., 2000; Li et al., 2000; MacLachlan et al., 2000; Sheikh et al., 2000), important in cell cycle arrest and apoptosis in response to cell stress (Sheikh et al., 2000; Taylor and Stark, 2001). BRCA1 functions as a co-repressor of GADD45 transcription by way of interactions with a DNA-binding protein termed zinc finger and BRCA1- interacting protein with a KRAB domain 1, ZBRK1 (Zheng et al., 2000b), a member of the Krupple-associated box-zinc finger protein (KRAB-ZFP) family of transcription repressors (Collins et al., 2001). The latest evidence indicates that ZBRK1 has a BRCA1-independent repressor function by way of its KRAB domain, and a C-terminal BRCA1-dependent repressor activity, $ZBRK1_{5-9}$ (Tan et al., 2004).

Our goal is to develop a biosensor system to visualize transcription control by ZBRK1 and BRCA1 in single living and/or fixed cells. The rationale is to use integrated DNA binding sites to obtain real time, multiplex-based data. When operational, this system would be capable of documenting real time nuclear dynamics of ZBRK1/BRCA1-dependent chromatin modification systems, as cells mount transcriptional responses to genotoxins.

Body:

Included with this report is a PowerPoint Presentation for the recent Era of Hope meeting in Philadelphia. This is provided to document the methodology and preliminary results. Also appended is a presentation by a student, Tiffany Jones, who worked on this project as a rotation student in my laboratory.

Negative findings are that expression of the GFP-Gal4DBD has not been found to localize at fluorescent foci in the nuclei of any of our G-series cell lines. The expression of ZBRK1-GFP has also not resulted in any fluorescent nuclear foci in any of the Z-series lines. We have attempted this screen in live cells, which has lower microscopic resolution than fixed cells, which we will try next.

Finally, our first attempt at securing funding to keep this project going was not successful (see attached application for an Idea Award). We are currently looking for alternative funding sources. Since this is a technology development project, there have been, as yet, no publications.

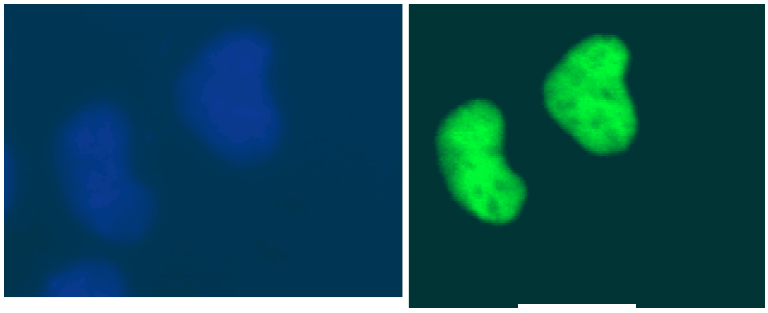
Key Research Accomplishments:

Key research accomplishments are the establishment of several cell lines (G-series and Z-series documented in the Body and appended PowerPoint files. We have also constructed a key screening reagent, GFP-GAL4-DBD, which is the green fluorescent protein fused to the DNA-binding domain of the GAL4 activator. Also, we have finished re-engineering ZBRK1-GFP fusion to a more modern enhanced green fluorescent protein.

Reportable Outcomes:

- UAS and ZRE array-bearing plasmids have been constructed.
- Transient and stable reporter expression have been demonstrated.
- Stable lines with G20, G40 and Z32 are on line for studies.
- Construction of fluorescent GAL4-DBD and ZBRK1 and BRCA1 derivatives are underway.

Data generated:



DAPI GFP

Figure 1. *ZBRK1-GFP expression plasmid.* In this experiment, a plasmid constructed by a student, Tiffany Jones, was being tested for functionality. The plasmid encodes a translational fusion of ZBRK1 and the green fluorescent protein (GFP). The purpose of constructing this plasmid is to test for binding to the Z-series clones containing multimerized ZBRK1-binding sites integrated into the chromosomes. DAPI staining identifies nuclei of three cells (one is partially in the field on the lower left) and green fluorescence in nuclei (right) identifies two transiently transfected cells, indicating the expression plasmid is working properly.

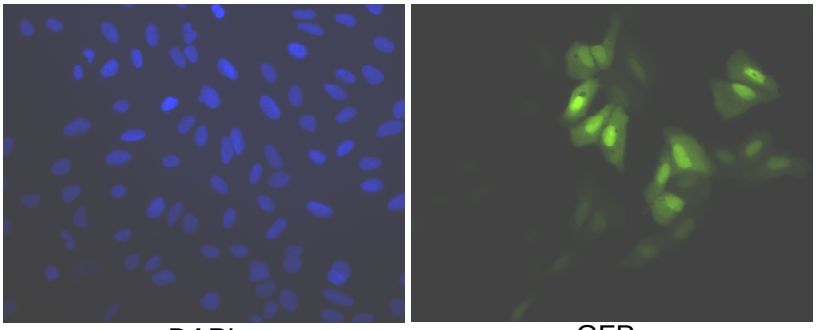


Figure 2. *GAL4-DBD-GFP expression plasmid.* In this experiment, aother plasmid constructed by a student, Tiffany Jones, was also being tested for functionality. The plasmid encodes a translational fusion of the GAL4 DNA-binding domain and the green fluorescent protein (GFP). The purpose of constructing this plasmid is to test for binding to the G-series (UAS) clones containing multimerized GAL4-binding sites integrated into the chromosomes. DAPI staining identifies nuclei of three cells (one is partially in the field on the lower left) and green fluorescence in nuclei (right) identifies two transiently transfected cells, indicating the expression plasmid is working properly. These images are taken from living cells.

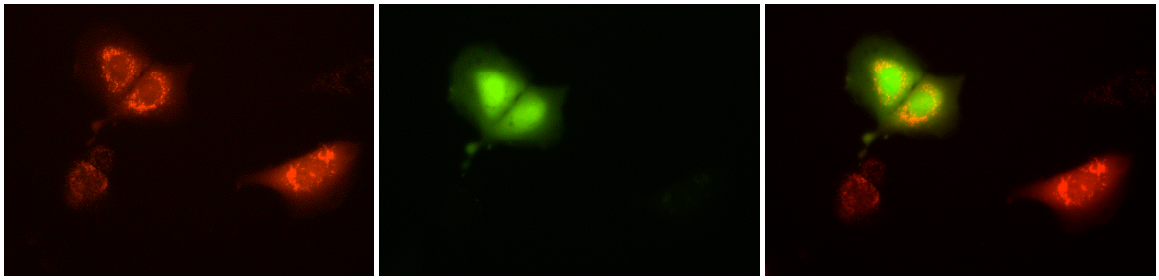


Figure 3. *GAL4-DBD-GFP expression in G-series cells*. In this experiment, we conducted the first test to determine if we could visualize a nuclear focus of green fluorescence in a one clonal line stably expressing dsRED in the cytoplasm (peroxisome targeted) and presumably bearing an integrated UAS GAL4 array of binding sites. None could be detected at this resolution or upon fixation and higher resolution analysis (not shown). On the left are dsRed expressing cells, which shows peroxisome-targeted fluorescence. The panel labeled GFP shows a transiently transfected cell expressing GFP-GAL4-DBD, which is predominantly nuclear in location. The right hand panel shows a merged image.

A bidirectional expression vector that contains a cDNA encoding BRCA1 translationally fused to GFP was also completed and sequenced for verification. Transient transfections will be used to verify the functionality of this construct.

Conclusion:

We envision our array-bearing lines as single cell biosensors for responses to DNA damage and other macromolecular trauma. For the first time it will be possible to obtain multiplex data both in real time and in high resolution fixed cell experiments. Thus, we can begin putting together a comprehensive picture of the high precision chromatin transactions involving ZBRK1 and BRCA1. This novel approach also has the potential to be adapted for other types of damage response transactions including repair and recombination. Finally, we think our cells will be extremely useful in high throughput microscopic screening that is currently coming on line in several laboratories (Perlman et al., 2004) using image cytometry. This could be important in identifying new compounds that will either up or down regulate the genotoxic response system.

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